

[0100] The detection cartridge 59 can be operated as follows: about 20 μ L of amplicon from the amplification chamber 11 is transferred, as described by the transfer method above, for detection by the enzyme-linked DNA hybrid sensor cartridge. The latter is described in jointly-owned U.S. Application Publication No. 2003/0170881. The detection device 59 is placed into an i-STAT model 300 electrochemical analyzer (i-STAT Corporation, East Windsor, N.J.) or other like instrument or analyzer. The sensor cartridge can include multiple (e.g., 2 or 4 or any suitable number) amperometric sensors coated with specific DNA oligomers (oligonucleotides). For purposes of illustration and not limitation, the oligonucleotides can be 5'-biotinylated oligonucleotides with 3' amine derivatives, and they can have at both termini a phosphorothioate backbone. These oligonucleotides are chemically bound to carboxyl derived beads at their 3'-amine derivatives by covalently bonding onto the sensor surface using the EDAC reaction, as is well known by skilled artisans. One of the sensors is bound with the complementary single-stranded DNA oligomer to one of the single-stranded portions of the PCR primers, as a control. Also present within this cartridge can be a separate streptavidin-alkaline phosphatase conjugate (strep-ALP).

[0101] In a preferred exemplary embodiment, the PCR amplified product and strep-ALP conjugate dissolved into a single solution can be brought into contact with the DNA capture sensors. Alternatively, it should be noted that the PCR product can be contacted with the sensor first, followed by the conjugate. In a preferred exemplary embodiment, the double-stranded PCR products, including both single-stranded hybridization regions, bind to the capture region on the amperometric sensor. Binding of the alkaline phosphatase label can occur either in solution before capture of the PCR product or after it has bound to the bead. After a controlled period of time, such as from about 5 to about 15 minutes, and at a controlled temperature (e.g., preferably about 37° C.), the solution is moved out of the sensor region and delivered to a waste chamber within the detection cartridge 59. A wash solution, containing substrate for ALP, is brought over the sensor washing excess strep-ALP conjugate away from the sensor region. A trailing portion of the wash solution remains on the sensor and provides an electrogenic substrate for the ALP label. Note that in an alternative exemplary embodiment, a wash solution can be used first, followed by a second solution containing the substrate. Note also that where an optical sensor or other type of sensor is used, other appropriate substrates can be used. In a preferred exemplary embodiment, the measured current at the capture sensor is essentially directly proportional to the number of ALP labels present on the sensor. An adjacent amperometric sensor that is not coated with the complementary DNA binding sequence can be used as a control sensor to offset any non-specific binding of the ALP reagent on the sensors, thus improving the detection limit. Alternatively or additionally, a capture oligonucleotide with a sequence different from the complimentary DNA binding sequence can be used as a negative control.

[0102] For purposes of illustration and not limitation, the following examples provide information on the amplification and detection of specific genetic markers.

EXAMPLE 1

[0103]

PCR Amplification of Hemachromatosis (Hfe) C282Y allele and detection		
Oligo designa- tion	Sequence (5'→3')	Characteris- tics
Is083	/5Bio/C*CAGA/iBiodT/CACAATGA GGGGCTGATC*C/	Hfe Contra sequence
Is084	/A*CTTCATACACAACCTCCCGCG TTGCATAACT/iSpC3/CCCCTGGG GAAGAGCAGAGATATATGT*G/	Wt C282 SNP discriminat- ing primer with Sc com- plement
Is085	/G*CGGCGCGATGCGCCACCTGC CGC/iSpC3/CCCCTGGGGAAGAGC AGAGATTACGT*A/	Mut Y282 SNP discriminat- ing primer with anti-MBW complement
Is071	amino_modifier_C12-T20- GCGGCAGGTGGCGCATCGCGCC GC	MBW capture
Is028.L2	amino_modifier_C12-T20- AGTTATGCAACGCGGAGTTGT GTATGAAGT	Sc Capture with anti-Sc

[0104] Designations: 5Bio—5'-biotinylated base; iBiodT—internal dT biotinylated base; *—phosphorothioate backbone; T20-20 dTs in the sequence; Amino_modifier_C12—5' amino derivative; iSpC3—spacer/blocker phosphoramidite; Hfe—Hemachromatosis gene, Wt—wild type, Mut—mutant; SNP—single nucleotide polymorphism; MBW selected sequence; Sc selected sequence.

[0105] In a preferred embodiment, the detection device (also referred to as a universal detection cartridge or UDC) is manufactured with two biosensors with detectable sequences for MBW and Sc. In independent reactions, oligonucleotides is071 and is028.L2 are added to carboxylated beads and chemically linked using EDAC via techniques well known to those skilled in the art. These beads are printed on wafers at two independent locations that are manufactured with gold metal sensors using techniques described in, for example, jointly-owned U.S. Application Publication No. 2003/0170881 (the '881 application), the entire contents of which are incorporated by reference herein. In addition to the beads bound with capture synthetic oligonucleotides, another print on the same chip includes a streptavidin-alkaline phosphatase conjugate. The wafers are diced and chips assembled along with an Ag/AgCl reference chip into detection devices of the type described in the '881 application. The fluidic elements of these detection devices are similar in format to commercial blood testing cartridges sold by, for example, i-STAT Corporation for measuring cardiac troponin I (cTnI).